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DIALOG(R)File 351:Derwent WPI

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WPI Acc No: 1981-93634D/198151
Derivs. of ML-236B - which are cholesterol biosynthesis inhibitors useful in treating hypercholesterolaemia
Patent Assignee: SANKYO CO LTD (SANY)
Inventor: TANAKA M; TERAHARA A
Number of Countries: 014 Number of Patents: 027
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
GB 2077264	A	19811216	GB 8117450	A	19810608	198151	B
BE 889150	A	19811209				198152	
DE 3122499	A	19811224	DE 3122499	A	19810605	198201	
FR 2483912	A	19811211				198203	
SE 8103560	A	19820104				198203	
NL 8102737	A	19820104				198205	
DK 8102470	A	19820111				198206	
JP 57002240	A	19820107	JP 8076127	A	19800606	198207	
FI 8101762	A	19820129				198209	
JP 57050894	A	19820325	JP 80124385	A	19800908	198218	
JP 57108039	A	19820705	JP 80130311	A	19800919	198232	
JP 57067575	A	19820424	JP 80115483	A	19800822	198235	
US 4346227	A	19820824	US 82351975	A	19820224	198236	
CA 1150170	A	19830719				198335	
AT 8102567	A	19830915				198340	
US 4410629	A	19831018				198344	
GB 2077264	B	19840426				198417	
US 4448979	A	19840515				198422	
CH 655090	A	19860327				198617	
JP 86013699	B	19860415				198619	
DE 3122499	C	19871126				198747	
JP 87054476	B	19871116				198749	
SE 453389	B	19880201				198807	
JP 88021672	B	19880509				198822	
IT 1144598	B	19861029				198833	
JP 88048858	B	19880930				198843	
NL 191738	B	19960102	NL 812737	A	19810605	199607	

Priority Applications (No Type Date): JP 80130311 A 19800919; JP 8076127 A 19800606; JP 80115483 A 19800822; JP 80124385 A 19800908
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
GB 2077264	A		28		
NL 191738	B		17	C12P-007/62	

Abstract (Basic): GB 2077264 A

ML-236B derivs. of formula (I) ring-closed lactones, salts and esters are new: where R is a gp. (II) or (III): (I) are cholesterol biosynthesis inhibitors and are thus useful in treating hypercholesterolaemia.

(I) are prepd. by enzymatic hydroxylation of ML-236B, or ML- 236B carboxylic acid or its salt or ester (see US398140). Pref. the enzyme is provided by a strain of Mucor, Phizopus, zygorynchus, circinella, Actinomucor, Gongronella, Phyomyces, Mortierella, Pycnoporus, Rhizoctonia, Absidia, Cunninghamella, Syncephalastrum or Streptomyces. A partic. suitable microorganism is Mucor hiemalis F. hiemalis, which gives at least 90% conversion of ML-236B and its derivs.

Title Terms: DERIVATIVE; ML; CHOLESTEROL; BIOSYNTHESIS; INHIBIT; USEFUL; TREAT; HYPERCHOLESTEROLAEMIC
Derwent Class: B03; B05; D16
International Patent Class (Main): C12P-007/62
International Patent Class (Additional): A61K-031/22; A61K-031/365; C07C-059/90; C07C-069/03; C07C-069/22; C07D-309/30; C12P-017/06;

C12R-001/46
File Segment: CPI
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[redacted]

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8 Sep 1980
19 Sep 1980
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16 Dec 1981
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366 367 368 36Y 389 625
628 633 638 643 652 658
65X 662 66X 672 761
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TV
(56) Documents cited
None
(58) Field of search
C2C
(71) Applicants
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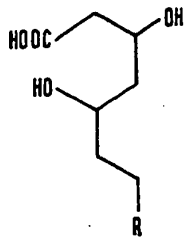
57—60 Lincoln's Inn
Fields, London WC2A 3LS

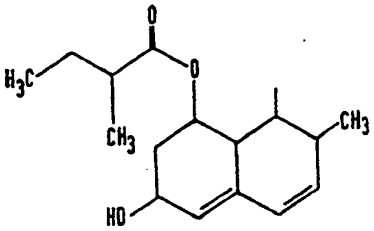
(54) ML-236B derivatives and their preparation

(57) Compounds of formula (I):

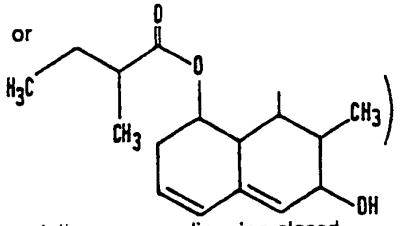


(I)

(wherein R represents a group of formula



or



and the corresponding ring-closed lactones, salts (especially alkali metal salts) and esters (especially C_1—C_5 alkyl esters) thereof may be prepared by subjecting ML-236B, or ML-236B carboxylic acid or a salt or ester thereof to enzymatic hydroxylation, which may be effected by means of microorganisms of the genera Mucor, Rhizopus, Zygorynchus, Circinella, Actinomucor, Gongronella, Phycomyces, Martierella, Pycnoporus, Rhizoctonia, Absidia, Cunninghamella, Syncephalosporum and Streptomyces, or cell-free, enzyme-containing extracts from said microorganisms. The compounds are capable of *inhibiting biosynthesis of cholesterol* and are thus useful in the treatment of *hypercholesteraemia*.

GB 2 077 264 A

Fig 1

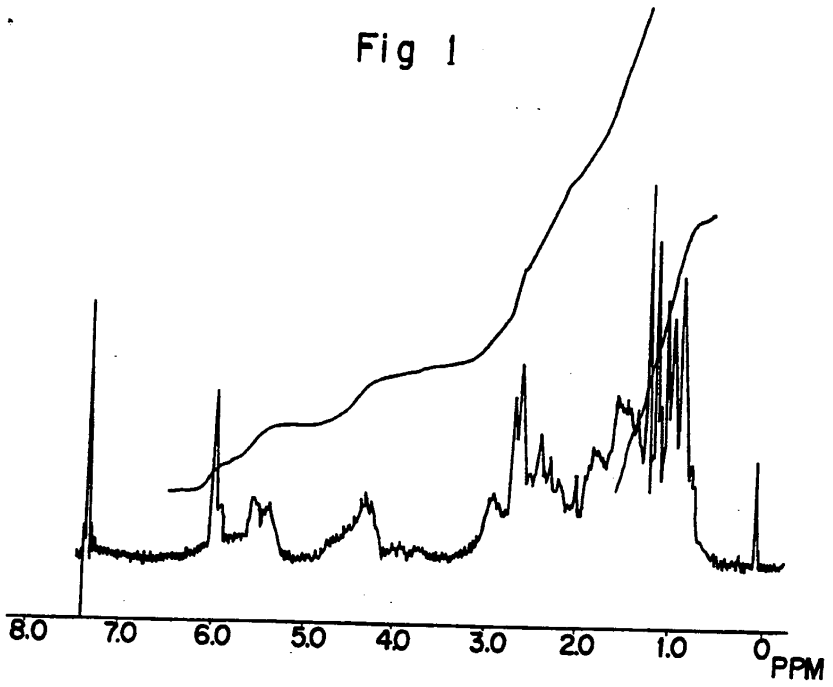


Fig 2

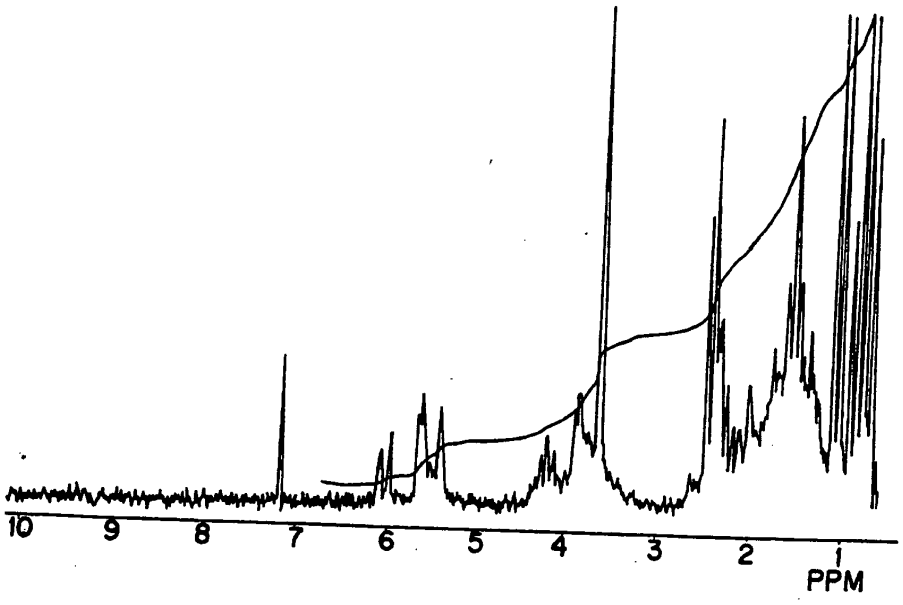


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Fig 3

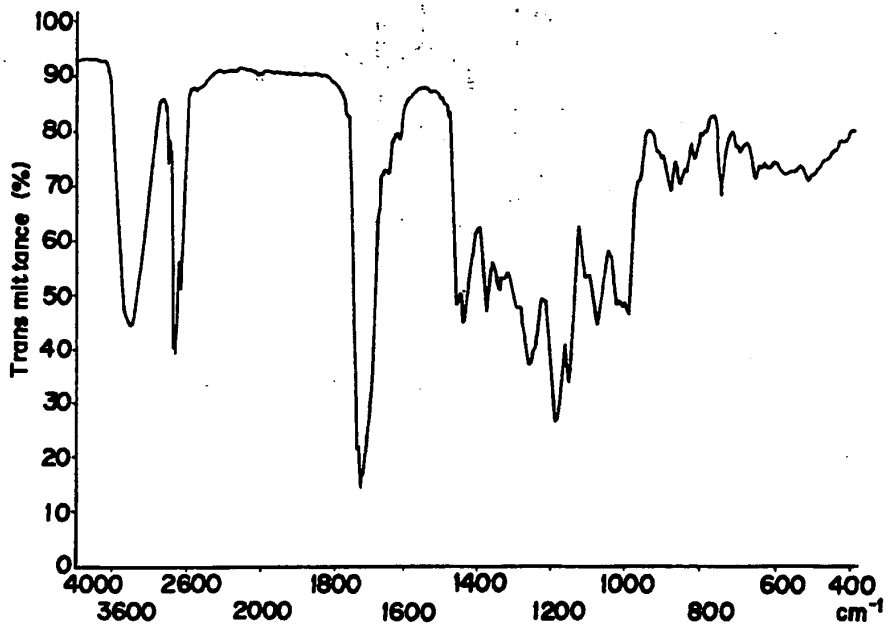


Fig 4

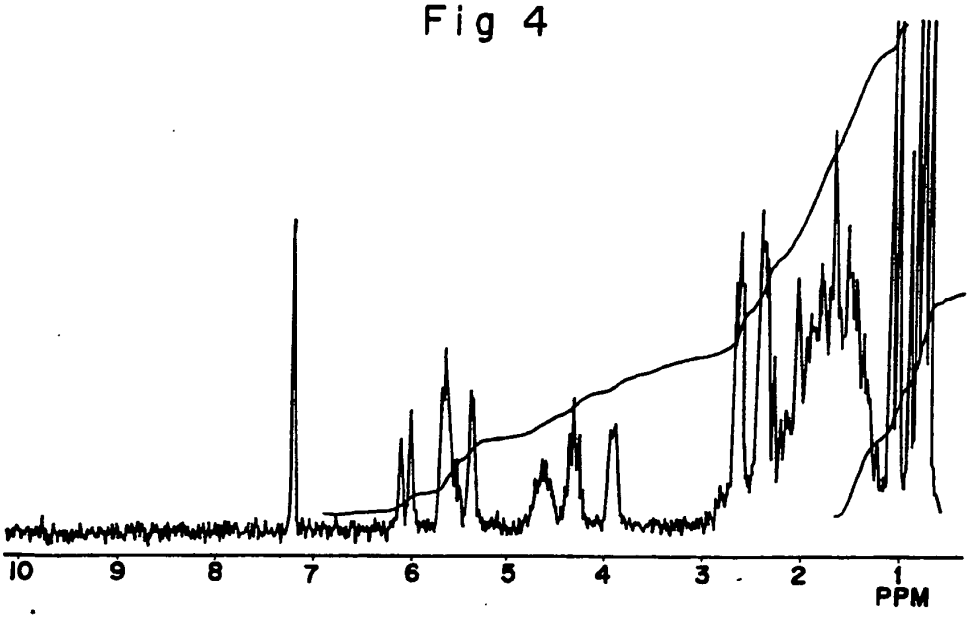
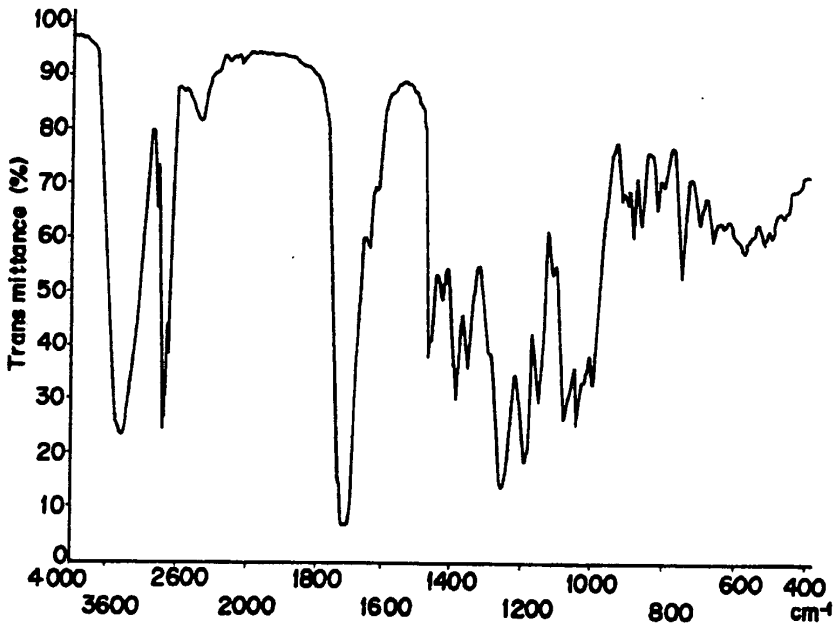


Fig 5

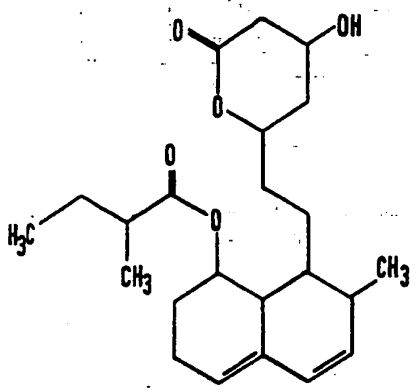

Trans mittance (%)
100
90
80
70
60
50
40
30
20
10
0
4000
3600
2600
2000
1800
1600
1400
1200
1000
800
600
400
cm-1

SPECIFICATION

ML—236B derivatives and th ir preparation

The present invention relates to a series of new derivatives of the known compound ML—236B, to processes for their preparation and to pharmaceutical compositions containing them.

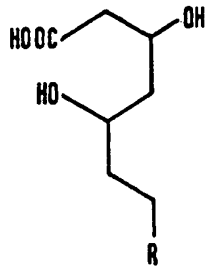
ML—236B, which has the following chemical structure:



is disclosed in U.S. Patent Specification No. 3,983,140. It has been isolated and purified from the metabolic products of microorganisms of the genus *Penicillium*, especially *Penicillium citrinum*, a species of blue mould. It has been shown to inhibit the biosynthesis of cholesterol by enzymes or cultured cells separated from experimental animals by competing with the rate-limiting enzyme active in the biosynthesis of cholesterol, namely 3-hydroxy-3-methylglutaryl-coenzyme A reductase and, as a result, significantly reduces serum cholesterol levels of animals [Journal of Antibiotics, *29*, 1346 (1976)]. A number of compounds structurally related to ML—236B have also been discovered and found to possess the ability to inhibit the biosynthesis of cholesterol.

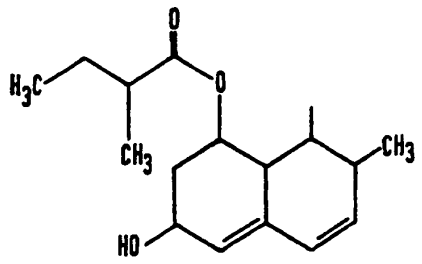
We have now discovered a series of new compounds, which may be prepared by the enzymatic hydroxylation of ML—236B or of derivatives thereof, and which possess an ability to inhibit the biosynthesis of cholesterol which is at least comparable with, and in some instances substantially exceeds, that of ML—236B itself.

The compounds of the present invention are those hydroxycarboxylic acids of formula (I):

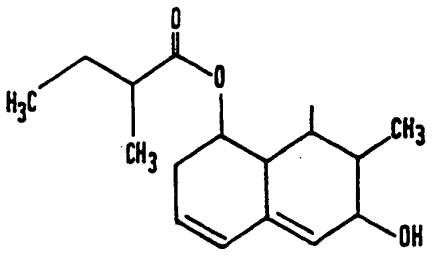


(I)

(in which R represents a group of formula



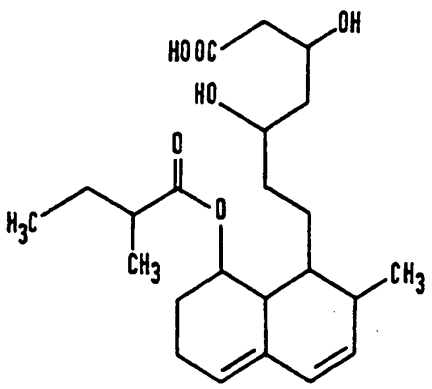
or



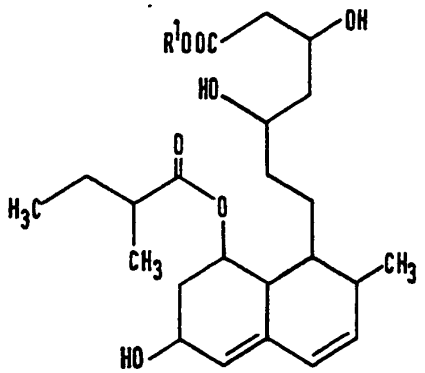
and ring-closed lact nes, salts and esters th reof.

The invention also provides a proc ss for preparing a compound of formula (I), r a ring-closed lact ne, salt or est r thereof by the enzymatic hydroxylation of ML—236B, or ML—236B carboxylic acid, r a salt r ester ther of.

ML—236B carboxylic acid has the formula

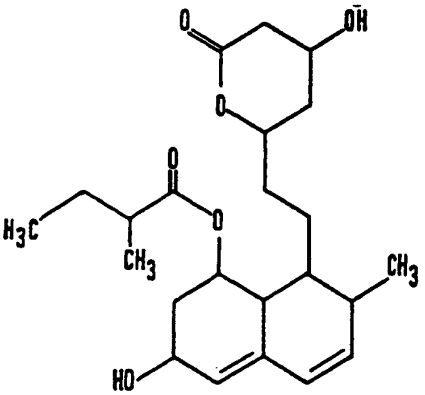


One class of compounds of the present invention are those compounds of formula (II):



(II)

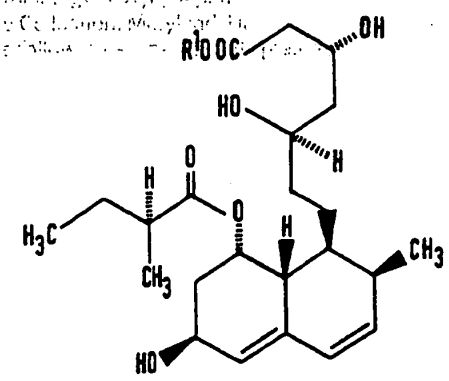
(in which R^1 represents a hydrogen atom or a C_1—C_5 alkyl group), pharmaceutically acceptable salts of the acid wherein R^1 represents a hydrogen atom, and the corresponding lactone of formula (III):



(III)

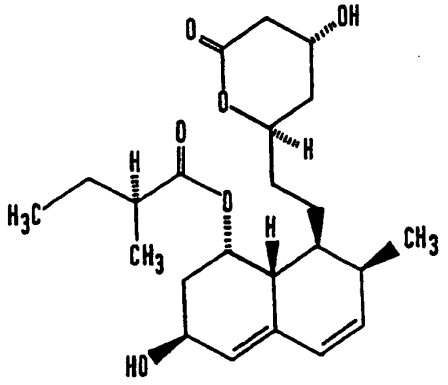
In view of the number of asymmetric carbon atoms in these compounds, a variety of geometric isomers are possible. Of these, the most important isomers are as follows:

Compounds of formula (IV):



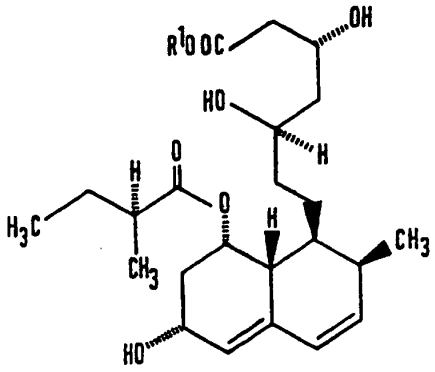
(IV)

(in which R^1 is as defined above) and pharmaceutically acceptable salts of the acid wherein R^1 represents a hydrogen atom, and the corresponding lactone of formula (V):



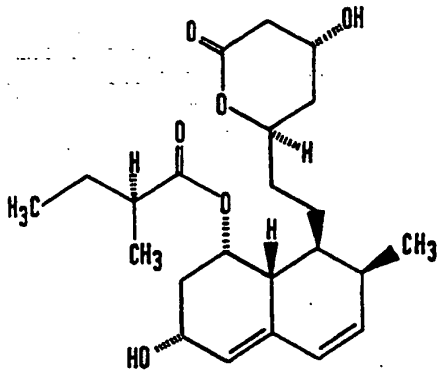
(V)

and compounds of formula (VI):



(VI)

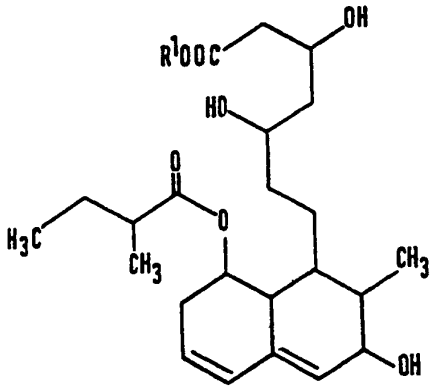
(in which R^1 is as defined above), and pharmaceutically acceptable salts of the acid wherein R^1 represents a hydrogen atom, and the corresponding lactone of formula (VII):



(VII)

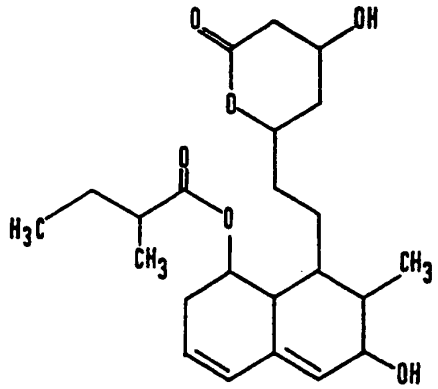
The hydroxy-carboxylic acid of formula (IV) in which R^1 represents a hydrogen atom is herein referred to as M—4 and derivatives of this acid, specifically the salts and esters, are named as derivatives of M—4, whilst the corresponding lactone of formula (V) is herein referred to as M—4 lactone. Similarly, the hydroxy-carboxylic acid of formula (VI) in which R^1 represents a hydrogen atom is referred to as M—4′ and derivatives of this acid are referred to as derivatives of M—4′, whilst the corresponding lactone of formula (VII) is referred to as M—4′ lactone.

Another preferred class of compounds of the invention are those compounds of formula (VIII):



(VIII)

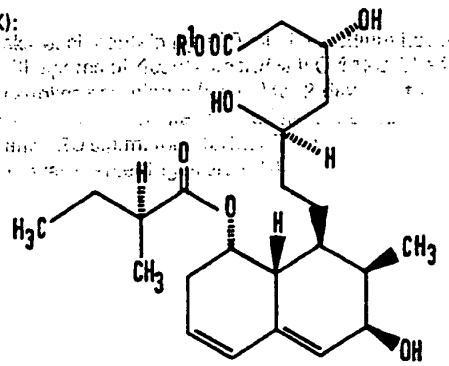
(in which R^1 is as defined above), and pharmaceutically acceptable salts of the acid in which R^1 represents a hydrogen atom, and the corresponding lactone of formula (IX):



(IX)

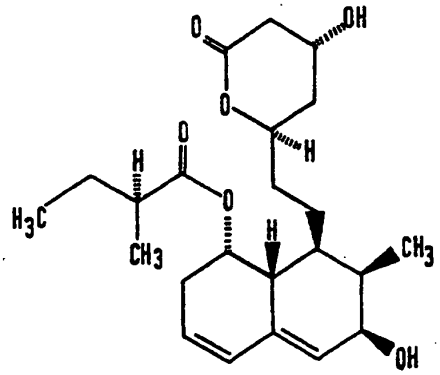
A variety of geometric isomers of these compounds are also possible, the most important being th following:

Compounds of formula (X):



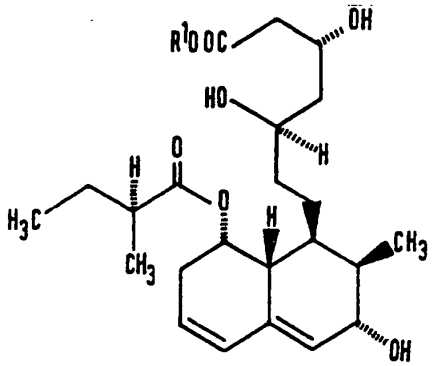
(X)

(in which R^1 is as defined above), and pharmaceutically acceptable salts of the acid in which R^1 represents a hydrogen atom and the corresponding lactone of formula (XI):



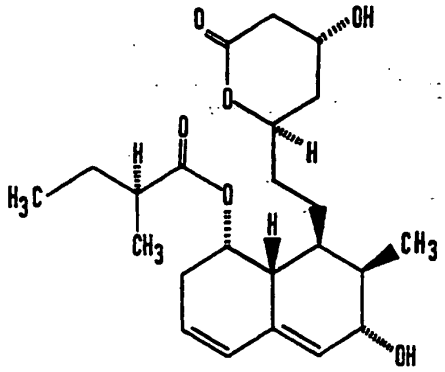
(XI)

and compounds of formula (XII):



(XII)

(in which R^1 is as defined above), and pharmaceutically acceptable salts of the acid in which R^1 represents a hydrogen atom and the corresponding lactone of formula (XIII):



(XIII)

The acid of formula (X) is herein referred to as IsoM—4 and its derivatives, such as salts and esters, are named as derivatives of IsoM—4, whilst the corresponding lactone of formula (XI) is herein referred to as IsoM—4 lactone. The acid of formula (XII) in which R^1 represents a hydrogen atom is herein referred to IsoM—4′, and its derivatives are named as derivatives of IsoM—4′, whilst its corresponding lactone of formula (XIII) is herein referred to as IsoM—4′ lactone.

Of the esters of the hydroxy-carboxylic acids of formula (I), the C_1—C_5 alkyl esters are preferred. These alkyl groups may be straight or branched-chain groups and include, for example the methyl, ethyl, propyl, isopropyl, butyl and isobutyl groups, of which the methyl group is particularly preferred.

The hydroxy-carboxylic acids will also form salts with a variety of cations, particularly metals and most preferably alkali metals, such as sodium or potassium. The sodium salts are most preferred.

Of the compounds of the invention, the most preferred compounds are M—4 lactone, M—4 sodium salt, M—4 methyl ester, IsoM—4′ lactone, IsoM—4′ sodium salt and IsoM—4′ methyl ester, M—4 sodium salt being particularly preferred.

The compounds of the invention may be prepared by the enzymatic hydroxylation of ML—236B or of a derivative thereof, specifically ML—236B carboxylic acid or a salt or ester thereof.

This enzymatic hydroxylation may be effected as part of the mammalian metabolism of ML—236B or a derivative thereof, for example by administering ML—236B to a suitable animal, collecting a metabolic product, e.g. urine, and then separating the desired compound or compounds of the invention from this metabolic product. Alternatively, the liver or an enzyme-containing extract from the liver may be used instead of the living animal. However, processes employing the animal metabolism or animal products have a relatively low productivity and are difficult to carry out reproducibly. Accordingly, we prefer to employ microorganisms or enzyme- containing extracts from their microorganisms.

Accordingly, the process of the present invention is preferably effected using a microorganism capable of converting ML—236B or a derivative thereof to a compound of the present invention or using an enzyme-containing extract of such a microorganism. Particularly preferred microorganisms are those of the following genera: Mucor, Rhizopus, Zygorynchus, Circinella, Actinomucor, Gongronella, Phycomyces, Martierella, Pycnoporus, Rhizoctonia, Absidia, Cunninghamella, Syncephalastrum and Streptomyces. In particular the following species are preferred:

Absidia coerulea
Cunninghamella echinulata
Syncephalastrum racemosum
Streptomyces roseochromgenus
Mucor hiemalis f. hiemalis
Mucor bacilliformis
Mucor circinelloides f. circinelloides
Mucor hiemalis f. corticolus
Mucor dimorphosporus
Mucor fragilis
Mucor genevensis
Mucor globosus
Mucor circinelloides f. griseo-cyanus
Mucor heterosporus
Mucor spinescens
Rhizopus chinensis
Rhizopus circinans
Rhizopus arrhizus
Zygorynchus moelleri
Circinella muscae
Circinella rigida

Circinella umbellata
Actinomucor elegans
Phycomyces blakesleeanus
Martierella isabellina
Gongronella butleri
Pycnoporus coccineus
Rhizoctonia solani
Syncephalastrum nigricans
Absidia glauca var. paradoxa

Amongst strains of the above species, the following are particularly preferred:

Absidia coerulea IFO—4423
Cunninghamella echinulata IFO—4445
Cunninghamella echinulata IFO—4444
Cunninghamella echinulata ATCC—9244
Syncephalastrum racemosum IFO—4814
Syncephalastrum racemosum IFO—4828
Streptomyces roseochromogenus NRRL—1233
Streptomyces roseochromogenus IFO—3363
Streptomyces roseochromogenus IFO—3411
Mucor hiemalis f. hiemalis IFO—5834
Mucor hiemalis f. hiemalis IFO—5303
Mucor hiemalis f. hiemalis IFO—8567
Mucor hiemalis f. hiemalis IFO—8449
Mucor hiemalis f. hiemalis IFO—8448
Mucor hiemalis f. hiemalis IFO—8565
Mucor hiemalis f. hiemalis CBS—117.08
Mucor hiemalis f. hiemalis CBS—109.19
Mucor hiemalis f. hiemalis CBS—200.28
Mucor hiemalis f. hiemalis CBS—242.35
Mucor hiemalis f. hiemalis CBS—110.19
Mucor hiemalis f. hiemalis CBS—201.65
Mucor bacilliformis NRRL—2346
Mucor circinelloides f. circinelloides IFO—4554
Mucor circinelloides f. circinelloides IFO—5775
Mucor hiemalis f. corticolus NRRL—12473
Mucor dimorphosporus IFO—4556
Mucor fragilis CBS—236.35
Mucor genevensis IFO—4585
Mucor globosus NRRL 12474
Mucor circinelloides f. griseo-cyanus IFO—4563
Mucor heterosporus NRRL—3154
Mucor spinescens IAM—6071
Rhizopus chinensis IFO—4772
Rhizopus circinans ATCC—1225
Rhizopus arrhizus ATCC—11145
Zygorynchus moelleri IFO—4833
Circinella muscae IFO—4457
Circinella rigida NRRL—2341
Circinella umbellata NRRL—1713
Circinella umbellata IFO—4452
Circinella umbellata IFO—5842
Phycomyces blakesleeanus NRRL—12475
Martierella isabellina IFO—6739
Gongronella butleri IFO—8080
Pycnoporus coccineus NRRL—12476
Rhizoctonia solani NRRL—12477
Syncephalastrum nigricans NRRL—12478
Syncephalastrum nigricans NRRL—12479
Syncephalastrum nigricans NRRL—12480
Absidia glauca var. *paradoxa* IFO—4431
Actinomucor elegans ATCC—6476

The microorganisms listed above are available from International Culture Collections, as indicated by the codes appended to their accession numbers, which codes have the following meanings.

IFO = Institute for Fermentation, Osaka, Japan
NRRL = Agricultural Research Culture Collection, Illinois, USA

CBS = Centraal Bureau voor Schimmelcultures, Netherlands
IAM = Institute f Applied Microbiology, Tokyo, Japan
ATCC = American Type Culture Coll ction, Maryland, USA.
Of the sp cies noted above, the following are particularly preferred:
Absidia coerulea
Cunninghamella echinulata
Syncephalastrum racemosum
Mucor hiemalis f. hiemalis
Mucor bacilliformis
Mucor circinelloides f. circinelloides
Mucor hiemalis f. corticolus
Mucor dimorphosporus
Mucor fragilis
Mucor genevensis
Mucor globosus
Mucor circinelloides f. griseo-cyanus
Mucor heterosporus
Mucor spinescens
Pycnoporus coccineus
Rhizoctonia solani
Syncephalastrum nigricans
and the following are particularly preferred strains of the species:
Absidia coerulea IFO—4423
Cunninghamella echinulata IFO—4445
Cunninghamella echinulata IFO—4444
Cunninghamella echinulata ATCC—9244
Syncephalastrum racemosum IFO—4814
Syncephalastrum racemosum IFO—4828
Mucor hiemalis f. hiemalis IFO—5834
Mucor hiemalis f. hiemalis IFO—5303
Mucor hiemalis f. hiemalis IFO—8567
Mucor hiemalis f. hiemalis IFO—8449
Mucor hiemalis f. hiemalis IFO—8448
Mucor hiemalis f. hiemalis IFO—8565
Mucor hiemalis f. hiemalis CBS—117.08
Mucor hiemalis f. hiemalis CBS—109.19
Mucor hiemalis f. hiemalis CBS—200.28
Mucor hiemalis f. hiemalis CBS—242.35
Mucor hiemalis f. hiemalis CBS—110.19
Mucor hiemalis f. hiemalis CBS—201.65
Mucor bacilliformis NRRL—2346
Mucor circinelloides f. circinelloides IFO—4554
Mucor circinelloides f. circinelloides IFO—5775
Mucor hiemalis f. corticolus NRRL—12473
Mucor dimorphosporus IFO—4556
Mucor fragilis CBS—236.35
Mucor genevensis IFO—4585
Mucor globosus NRR 12474
Mucor circinelloides f. griseo-cyanus IFO—4563
Mucor heterosporus NRRL—3154
Mucor spinescens IAM—6071
Pycnoporus coccineus NRRL—12476
Rhizoctonia solani NRRL—12477
Syncephalastrum nigricans NRRL—12478
Syncephalastrum nigricans NRRL—12479
Syncephalastrum nigricans NRRL—12480

For the preparation of compounds of formulae (IV) and (V) and their salts, the following species are preferred:
Mucor hiemalis f. hiemali
Mucor circinelloides f. circinelloides
Mucor fragilis
Mucor genevensis
Mucor circinelloides f. griseo-cyanus
Pycnoporus coccineus
Rhizoctonia solani.

For the preparation of compounds of formula (VI) and (VIII) and their salts, the species *Syncephalastrum nigricans* and *Syncephalastrum racemosum* are preferred.

For the preparation of compounds of formula (VIII) and (IX) and their salts, the species *Absidia coerulea* and *Cunninghamella echinulata* are preferred.

Of all of the species listed above, *Mucor hiemalis f. hiemalis* is particularly preferred since it is able to convert ML—236B and its derivatives to the desired compounds of formula (I) at a conversion of 90% or even higher.

Conversion of ML—236B or derivatives thereof to compounds of formula (I) may be achieved by contacting the complete cellular microorganism or, in some cases, a cell-free extract from the microorganism with ML—236B or a derivative thereof. The form of the compound produced will depend upon the culture conditions and the form of microorganism employed. Thus, for example, if the complete cellular microorganism is cultivated in the presence of ML—236B or a derivative thereof, the product will be the carboxylic acid, the lactone or alkali metal salt, depending upon the culture conditions, particularly the pH. On the other hand, if the ML—236B or derivative thereof is simply contacted with a resting cellular system or with a cell-free extract, the compound of the invention is obtained in the form of an alkali metal salt.

The progress of the conversion reaction may be determined by assaying samples of the reaction mixture during the course of the reaction to determine the degree of conversion. For example, the presence of M—4 lactone may be assayed by liquid chromatography employing as a carried Micro Bondapak C_{18} (manufactured by Waters Co. USA) and as the solvent 62% v/v aqueous methanol at the rate of 1 ml/minute. When detected using its ultraviolet absorption at 237 nm, M—4 gives a peak at a retention time of 10 minutes, and this may be used for the assay. Similar techniques are available for assaying the other compounds of the invention.

Where the microorganisms are to be cultivated in the presence of ML—236B or a derivative thereof to product the compounds of the invention, the culture conditions and culture media employed will be chosen having regard to the particular microorganism to be cultivated. Since the species of microorganism proposed for use in the process of the present invention are well known, culture conditions and culture media for use with these microorganisms are also well known.

The compounds of the invention may be separated from the reaction mixture by conventional means, for example by filtering off microbial cells (if necessary) and then subjecting the remaining mixture to any combination of thin layer chromatography, column chromatography or high performance liquid chromatography. The various compounds of the invention, where two or more are prepared together, may be separated from each other in the course of one or more of these chromatographic purification steps.

In addition to the compounds of the invention, there may, in some cases, also be prepared a compound which we have designated M—3 and which is known under the name 3′,5′-dihydroxy-(dihydro-ML—236B) in a copending application entitled "Hydronaphthalene Derivatives, their Preparation and Use". This may also be separated in the same way.

We have found that the compounds of the invention give a 50% inhibition of cholesterol biosynthesis at concentrations comparable with, or, in some cases, significantly less than, the concentrations required by ML—236B and certain other similar known compounds. The inhibitory activities of certain of the compounds of the invention, in terms of the concentration in μg/ml required to inhibit cholesterol biosynthesis by 50% [measured by the method described in the Journal of Biological Chemistry, *234*, 2835 (1959)] are as follows:

M—4 methyl ester	0.001
M—4 sodium salt	0.0008
M—4 lactone	0.016
IsoM—4′ methyl ester	0.007
IsoM—4′ lactone	0.013
M—4[1]	0.019
M—4[1] sodium salt	0.00049
ML—236B	0.01

The invention is further illustrated by the following Examples.

EXAMPLE 1

Preparation of M—4 lactone

Twenty 500 ml Sakaguchi flasks, each containing 100 ml of a medium having the composition described below, were inoculated with spores of *Absidia coerulea* IFO 4423. The flasks were subjected to shaking culture at 26°C and 120 strokes per minute (s.p.m.) for 2 days. At the end of this time, the sodium salt of ML—236B was added to each of the flasks to a final concentration of 0.05% w/v. Cultivation was continued at 26°C and 120 s.p.m. for a further 5 days.

The composition of the medium was (percentages are w/v):

Glucose	2.0%
K_2HPO_4	0.15%
$MgSO_4.7H_2O$	0.15%
NH_4NO_3	0.1%
Peptone	0.1%
Corn steep liquor	0.2%
Yeast extract	0.1%
$ZnSO_4.7H_2O$	0.001%
Tap water	the balance (adjusted to pH 7.0).

After completion of the cultivation, the reaction liquor was filtered, and the filtrate was adjusted with trifluoroacetic acid to pH 3. The resulting mixture was extracted with three 1 litre portions of ethyl acetate, to give extracts containing M—4. This compound shows an Rf value of 0.45 on thin layer chromatography (TLC) (Plate: Merck silica gel Art 5715; solvent: a 50:50:3 by volume mixture of benzene, acetone and acetic acid). The combined extracts were washed with saturated aqueous sodium chloride, and then a catalytic amount of trifluoroacetic acid was added for lactonization. The resulting mixture was then washed with a 1% w/v aqueous solution of sodium bicarbonate, dried over anhydrous sodiums sulphate and evaporated under reduced pressure to dryness. The residue was subjected to preparative liquid chromatography, System 500 using a Prep PAK—500/C_{18} cartridge manufactured by Waters Associates (Prep PAK is a Trade Mark). Purification with a 55% v/v aqueous methanol system yielded 50.1 mg of M—4 lactone.

M—4 lactone has the following physical properties.

1) Nuclear Magnetic Resonance Spectrum:

The NMR spectrum measured at 60 MHz in deuterochloroform using tetramethylsilane as the internal standard is shown in Figure 1 of the accompanying drawings.

2) Ultraviolet absorption spectrum (methanol solution) λ_{max}nm: 230; 236.7; 244.6.

3) Infrared absorption spectrum (liquid film) ν cm^{-1}: 3400, 2950, 1725.

4) Thin layer chromatography:

TLC plate: Merck silica gel Art 5715;

Solvent: benzene, acetone, acetic acid (50:50:3 by volume);

Rf value: 0.62.

EXAMPLE 2

48 mg of M—4 lactone were prepared following the same procedures as in Example 1, but using *Cunninghamella echinulata* IFO 4445.

EXAMPLE 3

30 mg of M—4 lactone were prepared following the same procedures as in Example 1, but using *Streptomyces roseochromogenus* NRRL 1233.

EXAMPLE 4

5 mg of M—4 lactone were prepared following the same procedures as in Example 1, but using *Syncephalastrum racemosum* IFO 4814.

EXAMPLE 5

6 mg of M—4 lactone were prepared following the same procedures as in Example 1, but using *Syncephalastrum racemosum* IFO 4828.

EXAMPLE 6

Preparation of IsoM—4′ methyl ester

Twenty 500 ml Sakaguchi flasks, each containing 100 ml of a medium having the composition described below, were inoculated with spores of *Absidia coerulea* IFO 4423. The flasks were subjected to shaking culture at 120 s.p.m. and 26°C for 2 days. At the end of this time, the sodium salt of ML—236B was added to each of the flasks to a final concentration of 0.05% w/v. Cultivation was continued at 120 s.p.m. and 26°C for a further 5 days.

The composition of the medium was (percentages are w/v):

Glucose	2.0%
K_2HPO_4	0.15%
$MgSO_4.7H_2O$	0.15%
NH_4NO_3	0.1%
Peptone	0.1%
Corn steep liquor	0.2%
Yeast extract	0.1%
$ZnSO_4.7H_2O$	0.001%
Tap water	the balance (adjusted to pH 7.0).

After completion of the cultivation, the reaction liquor was filtered, and the filtrate was adjusted with trifluoroacetic acid to pH 3. The resulting mixture was extracted with three 1 litre portions of ethyl acetate to give extracts containing IsoM—4′. This compound has an Rf value of 0.45 on thin layer chromatography (plate: Merck silica gel Art 5715; solvent: a 50:50:3 by volume mixture of benzene, acetone and acetic acid). The extract was washed with a saturated aqueous solution of sodium chloride, and then an ethereal solution of diazomethane was added. The mixture was allowed to stand for 30 minutes and then evaporated under reduced pressure to dryness. The residue was placed on a Lobar column (Merck Si 60, Size A) and purified using as the solvent system a 1:1 by volume mixture of benzene and ethyl acetate. There were obtained 200 mg of an IsoM—4′ methyl ester fraction. This fraction was further purified on a Lobar column (Merck RP—8, Size A) using 35% v/v aqueous acetonitrile as the eluent to give 78 mg of pure IsoM—4′ methyl ester, having the following characteristics:

1) Nuclear Magnetic Resonance Spectrum:

The NMR spectrum measured at 100 MHz in deuterochloroform using tetramethylsilane as the internal standard is shown in Figure 2 of the accompanying drawings.

2) Mass spectrum:

Measurement was made [after silylation with *N,O*-bis(trimethylsilyl)trifluoroacetamide] using a mass spectrometer, type D—300 manufactured by Nippon Electronics.

M/e: 654 (M^+), 552, 462, 372, 272, 233, 231.

3) Ultraviolet absorption spectrum (methanol solution) λ_{max}nm: 229, 234.8; 244.5.

4) Infrared absorption spectrum (liquid film): As shown in Figure 3 of the accompanying drawings.

5) Thin layer chromatography:

TLC plate: Merck silica gel Art 5715;

Solvent: benzene, acetone (1:1 by volume);

Rf value: 0.88.

By operating as described above but replacing the diazomethane by another appropriate diazoalkane, it is possible to produce other esters of IsoM—4′.

EXAMPLE 7

Preparation of IsoM—4′ lactone

The procedure described in Example 6 was repeated up to and including extraction with ethyl acetate to give extracts containing IsoM—4′. The combined extracts were washed with a saturated aqueous solution of sodium chloride and then evaporated to dryness to give the lacton product. The resulting residue was placed on a Lobar column (Merck Si 60, Size A) and purified using as the solvent system a 1:1 by volume mixture of benzene and ethyl acetate, to afford 198 mg of IsoM—4′ lacton.

This product was further purified by means of a Lobar column (Merck RP—8, Size A) eluted with 35% v/v aqueous acetonitrile, to give 82 mg of pure IsoM—4 lactone, having the following characteristics:

1) Nuclear Magnetic Resonance Spectrum:

The NMR spectrum measured at 100 MHz in deuterochloroform using tetramethylsilane as the internal standard is shown in Figure 4 of the accompanying drawings.

2) Ultraviolet absorption spectrum (methanol solution) λ_{max}nm: 229, 234.8; 244.5.

3) Infrared absorption spectrum (liquid film): As shown in Figure 5 of the accompanying drawings.

EXAMPLE 8

63 mg of IsoM—4′ lactone were prepared, following the same procedures as in Example 7, but using *Cunninghamella echinulata* IFO 4445.

EXAMPLE 9

24 mg of IsoM—4′ lactone were prepared, following the same procedures as in Example 7, but using *Syncephalastrum racemosum* IFO 4814.

EXAMPLE 10

35 mg of IsoM—4′ lactone were prepared, following the same procedures as in Example 7, but using *Syncephalastrum racemosum* IFO 4828.

EXAMPLE 11

12 mg of IsoM—4′ lactone were produced according to the process described in Example 7, but using *Streptomyces roseochromogenus* NRRL 1233.

EXAMPLE 12

Preparation of IsoM—4′ sodium salt

In a small amount of acetone were dissolved 10 mg of IsoM—4′ lactone. To the solution was added an equivalent amount of sodium hydroxide and the mixture was allowed to stand for 1 hour. The pH of the resulting mixture was adjusted with 0.1N hydrochloric acid to a value of 8.0. The acetone was then distilled off, and the residue was placed on an XAD—20 column (about 20 ml). The column was washed with distilled water and then eluted with 50 ml of 50% v/v aqueous acetone. The acetone was again distilled off, and the residue was freeze-dried to afford 6 mg of IsoM—4′ sodium salt, having the following characteristics:

1) Ultraviolet absorption spectrum (methanol solution) λ_{max}nm: 229 (shoulder); 235, 245 (shoulder).

2) Infrared absorption spectrum (KBr) ν cm^{-1}: 3400, 2850, 1710, 1580.

3) Thin layer chromatography:

TLC plate: Merck silica gel Art 5715;

Solvent: benzene, acetone, acetic acid (50:50:3 by volume);

Rf value: 0.45.

EXAMPLE 13

Preparation of M—4 methyl ester

Twenty 500 ml Sakaguchi flasks, each containing 100 ml of a medium of the same composition as shown in Example 1, were inoculated with spores of *Absidia coerulea* IFO 4423. The flasks were subjected to shaking culture at 26°C and 120 s.p.m. for 2 days. The sodium salt of ML—236B was then added to each of the flasks to a final concentration of 0.05 w/v. Cultivation was continued at 26°C and 120 s.p.m. for a further 5 days.

After completion of the cultivation, the reaction liquor was filtered, and the filtrate was adjusted with trifluoroacetic acid to pH 3. The resulting mixture was extracted with three 1 litre portions of ethyl acetate, to give extracts containing M—3, M—4 and IsoM—4′. Both M—4 and IsoM—4′ show an Rf value of 0.45 on thin layer chromatography (Plate: Merck silica gel Art 5715; solvent: a 50:50:3 by volume mixture of benzene, acetone and acetic acid). The combined extracts were washed with saturated aqueous sodium chloride, and then an ethereal solution of diazomethane was added. The mixture was allowed to stand for 30 minutes and then evaporated under reduced pressure to dryness. When the residue was placed on a Lobar column (Merck Si 60, Size A), and purification was effected using a 1:1 by volume mixture of benzene and ethyl acetate, a fraction containing IsoM—4′ methyl ester and a fraction containing M—4 methyl ester were separated. There were obtained 185.3 mg of the latter active fraction, from which 20 mg of pure M—4 methyl ester were obtained as a colourless oil by using a Lobar column (Merck RP—8, Size A) and eluting with 35% v/v aqueous acetonitrile.

M—4 methyl ester has the following characteristics:

1) Nuclear Magnetic Resonance Spectrum:

Measurement was made at 200 MHz in deuterochloroform using tetramethylsilan as the internal standard.

δ ppm:
0.88 (3H, triplet, J = 7.3 Hz);
0.89 (3H, doublet, J = 6.5 Hz);
1.12 (3H, doublet, J = 6.8 Hz);
1.1—1.7 (10H, multiplet);
2.34 (1H, sextuplet, J = 7 Hz);
2.3—2.5 (2H, multiplet);
2.49 (2H, doublet, J = 6.4 Hz);
2.58 (1H, multiplet);
3.72 (3H, singlet);
3.78 (1H, multiplet);
4.25 (1H, quintet, J = 7Hz);
4.4 (1H, multiplet);
5.42 (1H, multiplet);
5.56 (1H, multiplet);
5.90 (1H, doubled doublet, J = 9.8 and 5.6 Hz);
5.99 (1H, doublet, J = 9.8 Hz).

2) Mass spectrum:

Measurement was made [after silylation with *N,O*-bis(trimethylsilyl)trifluoroacetamide] using a mass spectrometer, type D—300 manufactured by Nippon Electronics.

M/e: 654(M^+), 552, 462, 372, 290, 272, 233, 231.

3) Ultraviolet absorption spectrum (ethanol solution) λ_{max}nm: 230.1; 237.3; 246.4.

4) Infrared absorption spectrum (liquid film) ν cm^{-1}: 3400, 2950, 1730.

5) Thin layer chromatography:

TLC plate: Merck silica gel Art 5715;
Solvent: benzene and acetone (1:1 by volume);
Rf value: 0.88.

By operating as described above but replacing the diazomethane by another appropriate diazoalkane, it is possible to produce other esters of M—4.

EXAMPLE 14

Preparation of Sodium Salts of M—4 and IsoM—4′

The procedure described in Example 1 was repeated (except that the culture medium contained Na_2HPO_4 instead of K_2HPO_4) up to and including filtration of the reaction liquor. The filtrate was then adsorbed on an HP—20 column (manufactured by Mitsubishi Chemical Industries). After washing the column with water, fractions containing M—4 sodium salt, IsoM—4′ sodium salt and M—3 sodium salt were eluted with 50% v/v aqueous acetone. The active fractions were freeze-dried, giving 830 mg of a freeze-dried product, which was purified by repeatedly subjecting it to high-performance liquid chromatography (column: Micro Bondapak C_{18}, 40% v/v aqueous methanol 1 ml/min.) to give 32 mg of M—4 sodium salt and 280 mg of IsoM—4′ sodium salt.

The properties of the IsoM—4′ sodium salt were identical to those of the product of Example 12 and the properties of the M—4 sodium salt are as follows:

1) Nuclear Magnetic Resonance Spectrum:

Measurement was made at 200 MHz in deuteromethanol using tetramethylsilane as the internal standard.

δ ppm:
0.91 (3H, triplet, J = 7.5 Hz);
0.92 (3H, doublet, J = 7 Hz);
1.12 (3H, doublet, J = 7 Hz);
1.1—1.8 (10H, multiplet);
2.25 (1H, doubled doublet, J = 15 and 7.6 Hz);
2.34 (1H, doubled doublet, J = 15 and 5.5 Hz);
2.2—2.4 (3H, multiplet);
2.48 (1H, multiplet);
3.68 (1H, multiplet);
4.07 (1H, multiplet);
4.28 (1H, multiplet);
5.36 (1H, multiplet);
5.48 (1H, doubled doublet, J = 3 and 2 Hz);
5.88 (1H, doubled doublet, J = 9.6 and 5.3 Hz);
5.98 (1H, doublet, J = 9.8 Hz).

2) Ultraviolet absorption spectrum (methanol solution) λ_{max}nm: 230.0; 237.2; 245.0.

3) Infrared absorption spectrum (KBr) ν cm^{-1}: 3400, 2900, 1725, 1580.

4) Thin layer chromatography:
TLC plate: Merck silica gel Art 5715;
Solvent: benzene, acetone and ac tic acid (50:50:3 by volume);
Rf value: 0.45.

EXAMPLE 15

18 mg of M—4 methyl ester were prepared, following the same procedures as in Example 13, but using *Cunninghamella echinulata* IFO 4445.

EXAMPLE 16

33 mg of M—4 methyl ester were prepared, following the same procedures as in Example 13, but using *Streptomyces roseochromogenus* NRRL 1233.

EXAMPLE 17

12 mg of M—4 methyl ester were prepared, following the same procedures as in Example 13, but using *Syncephalastrum racemosum* IFO 4814.

EXAMPLE 18

16 mg of M—4 methyl ester were prepared, following the same procedures as in Example 13, but using *Syncephalastrum racemosum* IFO 4828.

EXAMPLE 19

Preparation of M—4 methyl ester

Five beagles (male, average weight 10 kg) were administered with ML—236B at a dose of 200 mg/kg/day and their urine was collected for 3 days. 3 litres of collected urine were passed through a 500 ml XAD—2 column, eluted with 500 ml of 50% v/v aqueous acetone, and, after distilling off the acetone under reduced pressure, the residual liquid was adjusted to pH 3 by the addition of trifluoroacetic acid. The mixture was then extracted three times, each time with 1 litre of ethyl acetate, to give M—4. This compound shows an Rf value of 0.45 on thin layer chromatography (TLC plate: Silica Gel Art 5715 manufactured by Merck & Co., Inc.; solvent: a 50:50:3 by volume mixture of benzene, acetone and acetic acid). The extract was washed with a saturated aqueous solution of sodium chloride, and, after adding an ethereal solution of diazomethane, left standing for 30 minutes. It was then evaporated to dryness under reduced pressure. The residue was dissolved in 10 ml of a 55% v/v aqueous methanol solution, and passed through a column chromatograph (Product of Merck & Co., Inc.; RP—8, Size B). After passing 200 ml of a 55% v/v aqueous methanol solution, it was eluted with a 60% v/v aqueous methanol solution. The first 240 ml of the eluate were discarded, and the next 120 ml were collected. This fraction was evaporated to dryness and the residue was dissolved in 2.5 ml of a 65% v/v aqueous methanol solution and purified by high-performance liquid chromatography (JASCO—Trirotar, column: μ — Bondapak C_{18}). The portion which showed the fourth peak was separated and the solvent was distilled off to give M—4 methyl ester as a colourless oil having the properties shown in Example 13. 4 mg of product were obtained.

EXAMPLE 20

Preparation of M—4

Homogenized rabbit liver was used in this Example to obtain M—4 from ML—236B.

(a) Enzymatic solution

Three volumes of a 1.15% w/v potassium chloride — 10 mM phosphate (pH 7.4) buffer solution were added to one volume of rabbit liver and the mixture was homogenized. The homogenized mixture was then centrifuged for 20 minutes at 9,000 G and the supernatant fraction was taken as an enzymatic solution.

(b) Cofactor solution

β-Nicotinamide adenine dinucleotide phosphate (reduced form NADPH)	3 mg
$MgCl_2$ solution (508 mg/10 ml)	0.1 ml
1.15% w/v KCl solution	0.3 ml
0.2 M phosphate buffer solution (pH 7.4)	0.6 ml

The above substances wer mixed to a total volume of 1 ml to make the cofactor solution.

(c) Reaction solution

80 μl of the above enzymatic solution, 20 μl of the above cofactor solution and 2 μl of a methanol solution of ML—236B were mixed to make a final concentration of ML—236B of 1 mM. The resulting solution was shaken for 30 minutes at 37°C. M—4 was formed in the reaction mixture and identified by TLC (the same conditions as in Example 19).

EXAMPLE 21

Preparation of M—4 sodium salt

2 mg of M—4 methyl ester were dissolved in 1 ml of a 0.1 N aqueous solution of sodium chloride and subjected to hydrolyzation at 30°C for 1 hour. The reaction mixture was washed with 1 ml of chloroform and the resulting aqueous phase was adjusted to pH 8 with 0.1 N hydrochloric acid and passed through a XAD—2 column (about 5 ml). The column was washed with 20 ml of distilled water and the desired product was eluted with 15 ml of 50% v/v aqueous acetone. The acetone was distilled off from the eluate. The residue was confirmed by high-performance liquid chromatography to give a single peak (retention time was 13 minutes, eluted with 40% v/v aqueous methanol at 1 ml/minute). The residue was then lyophilized to give 0.8 mg of M—4 Na salt having the same properties as the product of Example 14.

EXAMPLE 22

Preparation of M—4 methyl ester

Each of twenty 500 ml. Erlenmeyer flasks containing 100 ml. of a medium having the composition listed below was inoculated with spores of *Mucor hiemalis f. hiemalis* IFO—5834. The inoculum was subjected to shaking culture at 26°C. and 220 rpm. After 4 days, ML—236B was added to a final concentration of 0.05% w/v, and cultivation was conducted at 26°C. and 220 rpm for additional 6 days. The composition of the medium was (percentages are w/v):

Glucose	1.0%
Peptone	0.2%
Meat extract	0.1%
Yeast extract	0.1%
Corn steep liquor	0.3%
Tap water	balance (pH unadjusted).

After completion of the cultivation, the filtrate was adjusted to a pH of 3 with trifluoroacetic acid. The mixture was then extracted three times, each time with 100 ml. of ethyl acetate. There was obtained a fraction containing M—4. M—4 has an Rf value of 0.45 on thin layer chromatography (Plate: Merck Silica gel Art 5715; Solvent: a 50:50:3 by volume mixture of benzene, acetone and acetic acid). The conversion ratio was 90%. This extract was washed with a saturated aqueous solution of sodium chloride, after which there was added an ethereal solution of diazomethane. The resulting mixture was allowed to stand for 30 minutes and then concentrated under reduced pressure to dryness. The residue was placed on a Lobar column (Merck Si 60, size A) and purified with a 1:1 by volume mixture of benzene and ethyl acetate. There were obtained about 600 mg. of M—4 methyl ester, having the same properties as the product of Example 13.

EXAMPLE 23

Preparation of M—4 lactone

The procedure described in Example 22 was repeated up to and including washing of the three ethyl acetate extracts with a saturated aqueous solution of sodium chloride. The resulting solution was then evaporated to dryness to give a lactone product. The product was recrystallized from ethyl acetate to give about 560 mg. (56%) of M—4 lactone, having the same properties as the product of Example 1.

EXAMPLE 24

Preparation of M—4 sodium salt

The procedure described in Example 22 was repeated to give 1.9 litres of the filtrate from the conversion reaction. This was extracted three times, each time with 1 litre of ethyl acetate to give fractions containing M—4. By immediately transferring these into a 5% w/v aqueous solution of sodium bicarbonate, there was obtained a fraction containing M—4 sodium salt. Then the M—4 sodium fraction was adjusted with 2N hydrochloric acid to a pH of 7.0 and adsorbed on an HP—20 column (manufactured by Mitsubishi Chemical Industries). Washing with water and elution with 50% v/v aqueous acetone gave a fraction containing M—4 sodium salt, from which there were obtained 570

mg. (52%) of a fr eze-dried product, having the properties described in Example 14.

EXAMPLE 25

Preparation of M—4

The procedure described in Example 22 was repeated, except that the following microorganisms were employed and the conversion to M—4 was as shown by the associated codes:

Microorganism:	Conversion to M—4
Mucor hiemalis f. hiemalis IFO—5303	+4
Mucor hiemalis f. hiemalis IFO—8567	+4
Mucor hiemalis f. hiemalis IFO—8449	+4
Mucor hiemalis f. hiemalis IFO—8448	+4
Mucor hiemalis f. hiemalis IFO—8565	+4
Mucor hiemalis f. hiemalis CBS—117.08	+4
Mucor hiemalis f. hiemalis CBS—109.19	+4
Mucor hiemalis f. hiemalis CBS—200.28	+4
Mucor hiemalis f. hiemalis CBS—242.35	+4
Mucor hiemalis f. hiemalis CBS—110.19	+4
Mucor hiemalis f. hiemalis CBS—201.65	+4
Mucor bacilliformis NRRL—2346	trace
Mucor circinelloides f. circinelloides IFO—4554	+1
Mucor circinelloides f. circinelloides IFO—5775	+1
Mucor hiemalis f. corticolus NRRL—12473	trace
Mucor dimorphosporus IFO—4556	trace
Mucor fragilis CBS—236.35	+1
Mucor genevensis IFO—4585	+1
Mucor globosus NRRL—12474	trace
Mucor circinelloides f. griseo-cyanus IFO—4563	+1
Mucor heterosporus NRRL—3154	trace
Mucor spinescens IAM—6071	trace
Mucor chinensis IFO—4772	trace
Rhizopus circinans ATCC—1225	+1
Rhizopus arrhizus ATCC—11145	+1
Zygorynchus moelleri IFO—4833	+1
Circinella muscae IFO—4457	+
Circinella rigida NRRL—2341	trace

Microorganism:	Conversion to M—4
Circinella umbellata NRRL—1713	+1
Circinella umbellata IFO—4452	+1
Circinella umbellata IFO—5842	+1
Actinomucor elegans ATCC—6476	+1
Phycomyces blakesleeanus NRRL—12475	trace
Martierella isabellina IFO—6739	trace
Gongronella butleri IFO—8080	+1
Pycnoporus coccineus NRRL—12476	+3
Rhizoctonia solani NRRL—12477	+2

The codes representing the conversions to M—4 have the following meanings:
trace = 0.5% or less
+1 = 0.5—5%
+2 = 5.0—10.0%
+3 = 10.0—30.0%
+4 = 70.0—90.0%.

EXAMPLE 26
Preparation of IsoM—4′ lactone

Each of twenty 500 ml. Sakaguchi flasks containing 100 ml. of a medium of the composition described in Example 22 was inoculated with spores of *Circinella muscae* IFO—4457. The inoculum was subjected to shaking culture at 26°C. and 220 spm. After 4 days, ML—236B was added to a final concentration of 0.05% w/v, and cultivation was conducted at 26°C. and 120 spm for a further 6 days.

After completion of the cultivation, the conversion reaction mixture was filtered, and the filtrate was adjusted with trifluoroacetic acid to a pH of 3.0. The mixture was then extracted three times, each time with 1 litre of ethyl acetate, giving a fraction containing IsoM—4′. This extract was washed with a saturated aqueous solution of sodium chloride and then evaporated to dryness. There was obtained a lactone product. The residue was placed on a Lobar column (Merck Si 60, size A) and purified with an ethyl acetate system, giving 12 mg. of IsoM—4′ lactone, having the properties described in Example 7.

EXAMPLE 27
Preparation of M—4′ lactone

Each of twenty 500 ml. Erlenmeyer flasks containing 100 ml. of a medium having the composition shown below was inoculated with spores of *Syncephalastrum nigricans* NRRL—12478. The inoculum was subjected to shaking culture at 26°C. and 20 rpm. After 3 days, ML—236B was added to a final concentration of 0.05% w/v and cultivation was carried out at 26°C. and 220 rpm.

The composition of the medium was as follows (percentages are w/v):

Glucose	1%
Peptone	0.2%
Meat extract	0.1%
Yeast extract	0.1%
Corn steep liquor	0.3% (pH unadjusted).

After completion of the cultivation, the conversion reaction mixture was filtered, and the filtrate was adjusted with trifluoroacetic acid to a pH of 3. The mixture was then extracted three times, each time with 1 litre of ethyl acetate to give a fraction containing M—4′, which has an Rf value of 0.46 in thin layer chromatography (Plate: Merck silica gel Art 5715; solvent: a 50:50:3 by volume mixture of

benz ne, acetone and acetic acid). This extract was washed with a saturated aqueous solution of sodium chloride, dried over anhydrous sodium sulphat and subj cted to lactonization by adding a catalytic am unt of trifluoroacetic acid. The resulting mixture was then washed with a 5% w/v aqueous soluti n of sodium bicarb nate, dehydrated with anhydrous sodium sulphate and vaporated to dryness. The residue was crystallized from ethyl acetate to give about 180 mg. of M—4′ lactone having the following physical properties:

1) Nuclear Magnetic Resonance Spectrum:

Measured in deuterochloroform at 100 MHz, using tetramethylsilane as the internal standard.

δ ppm:
6.01 (1H, doublet);
5.90 (1H, quartet);
5.75 (1H, multiplet);
5.50 (1H, multiplet);
4.60 (1H, multiplet);
4.25 (1H, multiplet).

2) Ultraviolet Absorption Spectrum (methanol) λ_{max}nm: 230, 237, 245.

3) Infrared Absorption Spectrum (KBr) ν cm^{-1}: 3500, 1720.

4) Mass spectrum: M/e: (406(M^+), 304, 286.

5) Optical rotation: $[\alpha]_D^{25} = +310.9°$ (c = 0.66, methanol).

6) Melting point: 141—143°C.

7) Elemental analysis:

Calculated : C, 67.95%; H, 8.43%

Found : C, 68.05%; H, 8.37%.

8) Thin Layer Chromatography:
TLC plate: Merck silica gel Art 5715.
Solvent: Benzene — acetone (1:1 by volume)
Rf value 0.64.

EXAMPLE 28

Preparation of M—4′ sodium salt

Following substantially the same cultivation procedures as in Example 27, there was obtained a conversion reaction mixture.

After completion of the cultivation, the conversion reaction mixture was filtered, and the filtrate was adjusted with trifluoroacetic acid to a pH of 3. It was then extracted three times, each time with 1 litre of ethyl acetate to give a fraction containing M—4′, which was washed with a saturated aqueous solution of sodium chloride and immediately thereafter passed into a 5% w/v aqueous solution of sodium bicarbonate, to give a fraction containing M—4′ sodium salt. The aqueous layer thus obtained was adjusted to pH 8.0 with 0.1 N hydrochloric acid and adsorbed on a Diaion HP 20 resin column (manufactured by Mitsubishi Chemical Industries). It was then eluted with 50% v/v aqueous acetone. The acetone was distilled off, and the residue was freeze-dried to give 1.41 g. of M—4′ sodium salt, having the following physical properties:

1) Nuclear Magnetic Resonance Spectrum:

Measured in deuterochloroform at 60 MHz, using tetramethylsilane as the internal standard.

δ ppm:
6.00 (1H, doublet);
5.95 (1H, quartet);
5.70 (1H, broad singlet);
5.50 (1H, broad singlet).

2) Ultraviolet Absorption Spectrum (methanol) λ_{max}nm: 230, 238, 246.

3) Infrared Absorption Spectrum (KBr) ν cm^{-1}: 3400, 2900, 1680.

EXAMPLE 29

Preparation of M—4′ methyl ester

Following substantially the same cultivation procedures as in Example 27, there was obtained a conversion reaction mixture.

After completion of the cultivation, the conversion mixture was filtered and the filtrate was adjusted with trifluoroacetic acid to a pH of 3. It was then extracted three times, each time with 1 litre of ethyl acetate. Th combined extracts w re washed with a saturated aqueous solution of sodium chloride and then an ethereal solution of diazomethane was added thereto. The resulting mixture was allowed to stand for 30 minutes and then concentrated under reduced pressure to dryn ss. The residue was purified using a Lober column (M rck RP—8, size A) and a 1:1 by volume mixture of benzene and acetone as the dev loping s lvent. There were obtained 150 mg. of M—4′ methyl st r as a colourless

oily substance, having the following properties:

1) Nuclear Magnetic Resonance Spectrum:

Measured in deuterochloroform at 60 MHz, using tetramethylsilan as the internal standard.

δ ppm:

6.01 (1H, doublet);
5.90 (1H, quartet);
5.75 (1H, broad singlet);
5.50 (1H, broad singlet);
3.70 (3H, singlet).

2) Ultraviolet Absorption Spectrum (methanol) λ_{max}nm: 230, 238, 246.

3) Infrared Absorption Spectrum (liquid film) ν cm^{-1}: 3400, 1730.

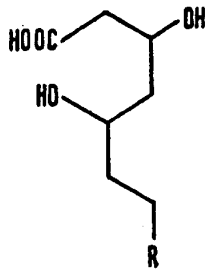
4) Mass analysis:

Measurement was made after silylation with *N,O*-bis(trimethylsilyl)trifluoroacetamide using a mass spectrometer, type D—300 manufactured by Nippon Electronics.

M/e: 654 (M^+).

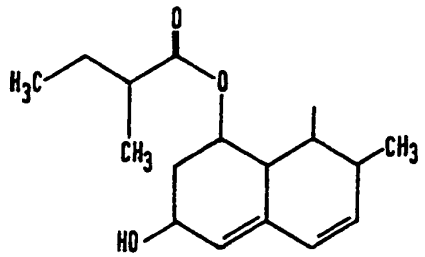
CLAIMS

1. Compounds of formula (I):

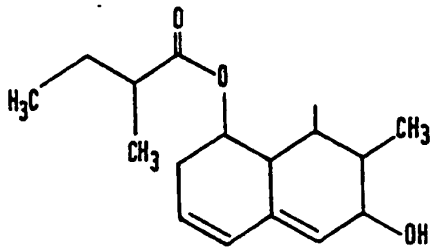


(I)

(in which R represents a group of formula

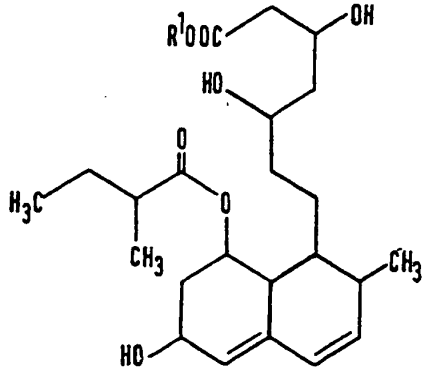


or



and ring-closed lactones, salts and esters thereof.

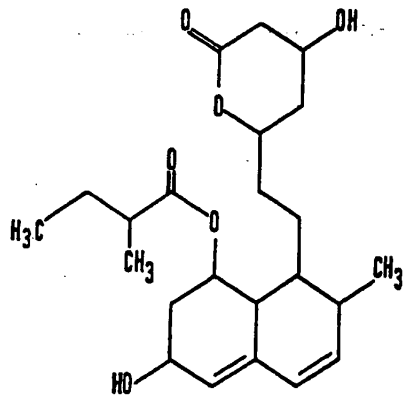
2. Compounds as claimed in Claim 1, having the formula (II):



(II)

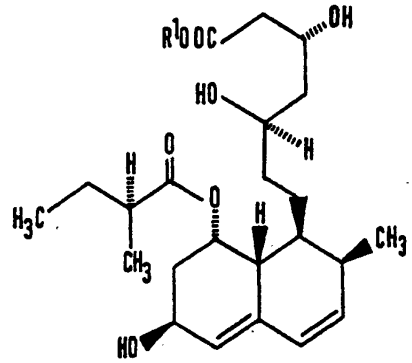
(in which R^1 represents a hydrogen atom or a C_1—C_6 alkyl group) and pharmaceutically acceptable salts of the acid wherein R^1 represents a hydrogen atom.

3. A compound as claimed in Claim 1, having the formula (III):



(III)

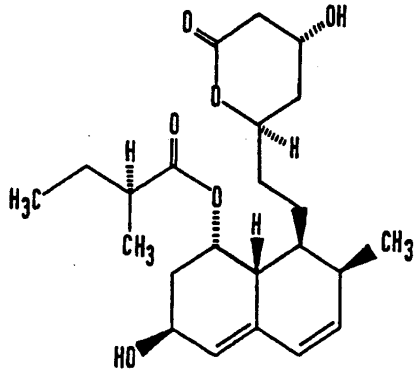
4. Compounds as claimed in Claim 2, having the configuration shown in formula (IV):



(IV)

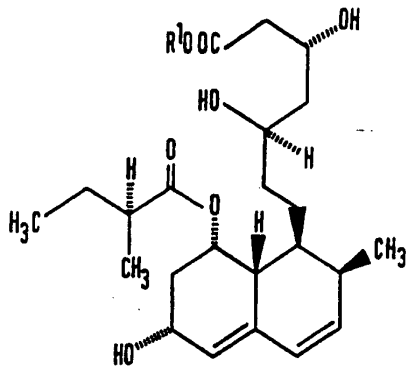
(wherein
R^1 is as defined in claim 2), and pharmaceutically acceptable salts thereof.

5. A compound as claimed in Claim 3, having the configuration shown in formula (V):



(V)

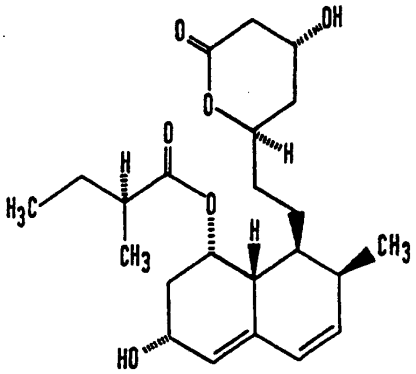
6. Compounds as claimed in Claim 2, having the configuration shown in formula (VI):



(VI)

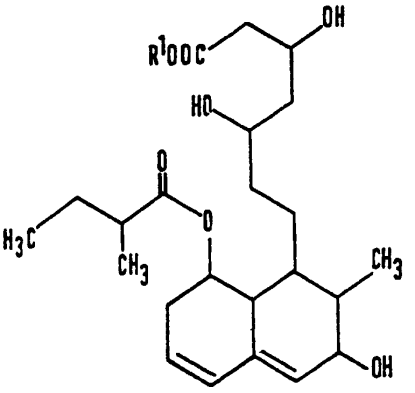
(wherein
R^1 is as defined in Claim 2) and pharmaceutically acceptable salts thereof.

7. A compound as claimed in Claim 3, having the configuration shown in formula (VII):



(VII)

8. Compounds as claimed in Claim 1, which have the formula (VIII):



(VIII)

(in which R^1 represents a hydrogen atom or a C_1—C_5 alkyl group) and pharmaceutically acceptable salts of the acid wherein R^1 represents a hydrogen atom.

9. Compounds as claimed in any one of Claims 2, 4, 6 and 8, wherein R^1 represents a hydrogen atom.

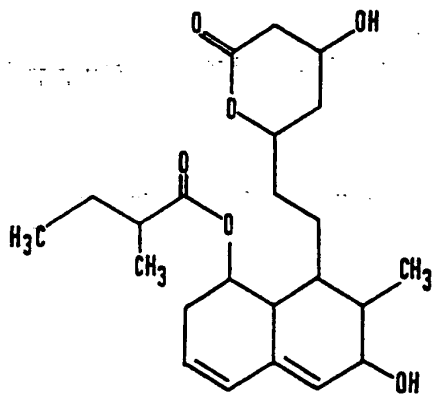
10. Compounds as claimed in any one of Claims 2, 4, 6 and 8, wherein R^1 represents a C_1—C_5 alkyl group.

11. Compounds as claimed in any one of Claims 2, 4, 6 and 8, wherein R^1 represents a methyl group.

12. Compounds as claimed in any one of Claims 2, 4, 6 and 8, in the form of the alkali metal salts.

13. Compounds as claimed in Claim 12, in the form of the sodium salt.

14. A compound as claimed in Claim 1, having the formula (IX):



(IX)

15. A process for preparing a compound as claimed in any one of the preceding claims, which comprises enzymatically hydroxylating ML—236B, or ML—236B carboxylic acid or a salt or ester thereof.

16. A process as claimed in Claim 15, wherein the enzymatic hydroxylation is effected by a microorganism of the genus Mucor, Rhizopus, Zygorynchus, Circinella, Actinomucor, Gongronella, Phycomyces, Martierella, Pycnoporus, Rhizoctonia, Absidia, Cunninghamella, Syncephalastrum or Streptomyces, or with a cell-free, enzyme-containing extract from said microorganisms.

17. A process as claimed in Claim 16, wherein said microorganism is:

Absidia coerulea
Cunninghamella echinulata
Syncephalastrum racemosum
Streptomyces roseochromogenus
Mucor hiemalis f. hiemalis
Mucor bacilliformis
Mucor circinelloides f. circinelloides
Mucor hiemalis f. corticolus
Mucor dimorphosporus
Mucor fragilis
Mucor genevensis
Mucor globosus
Mucor circinelloides f. griseo-cyanus
Mucor heterosporus
Mucor spinescens
Rhizopus chinensis
Rhizopus circinans
Rhizopus arrhizus
Zygorynchus moelleri
Circinella muscae
Circinella rigida
Circinella umbellata
Actinomucor elegans
Phycomyces blakesleeanus
Martierella isabellina
Gongronella butleri
Pycnoporus coccineus
Rhizoctonia solani
Syncephalastrum nigricans or
Absidia glauca var. paradoxa.

18. A process as claimed in Claim 17, wherein said microorganism is:

Absidia coerulea IFO—4423
Cunninghamella echinulata IFO—4445
Cunninghamella echinulata IFO—4444
Cunninghamella echinulata ATCC—9244
Syncephalastrum racemosum IFO—4814
Syncephalastrum racemosum IFO—4828
Streptomyces roseochromogenus NRRL—1233
Streptomyces roseochromogenus IFO—3363
Streptomyces roseochromogenus IFO—3411
Mucor hiemalis f. hiemalis IFO—5834

Mucor hiemalis f. hiemalis IFO—5303
Mucor hiemalis f. hiemalis IFO—8567
Mucor hiemalis f. hiemalis IFO—8449
Mucor hiemalis f. hiemalis IFO—8448
Mucor hiemalis f. hiemalis IFO—8565
Mucor hiemalis f. hiemalis CBS—117.08
Mucor hiemalis f. hiemalis CBS—109.19
Mucor hiemalis f. hiemalis CBS—200.28
Mucor hiemalis f. hiemalis CBS—242.35
Mucor hiemalis f. hiemalis CBS—110.19
Mucor hiemalis f. hiemalis CBS—201.65
Mucor bacilliformis NRRL—2346
Mucor circinelloides f. circinelloides IFO—4554
Mucor circinelloides f. circinelloides IFO—5775
Mucor hiemalis f. corticolus NRRL—12473
Mucor dimorphosporus IFO—4556
Mucor fragilis CBS—236.35
Mucor genevensis IFO—4585
Mucor globosus NRRL—12474
Mucor circinelloides f. griseo-cyanus IFO—4563
Mucor heterosporus NRRL—3154
Mucor spinescens IAM—6071
Rhizopus chinensis IFO—4772
Rhizopus circinans ATCC—1225
Rhizopus arrhizus ATCC—11145
Zygorynchus moelleri IFO—4833
Circinella muscae IFO—4457
Circinella rigida NRRL—2341
Circinella umbellata NRRL—1713
Circinella umbellata IFO—4452
Circinella umbellata IFO—5842
Phycomyces blakesleeanus NRRL—12475
Martierella isabellina IFO—6739
Gongronella butleri IFO—8080
Pycnoporus coccineus NRRL—12476
Rhizoctonia solani NRRL—12477
Syncephalastrum nigricans NRRL—12478
Syncephalastrum nigricans NRRL—12479
Syncephalastrum nigricans NRRL—12480
Absidia glauca var. paradoxa IFO—4431 or
Actinomucor elegans ATCC—6476

19. A process as claimed in any one of Claims 15 to 18, wherein there is separated from the reaction mixture one or more of M—4, M—4′, IsoM—4, IsoM—4′ or a salt, ester or lactone of M—4, M—4′ IsoM—4 or IsoM—4′.

20. A process as claimed in Claim 19, wherein said ester is a C_1—C_5 alkyl ester.

21. A process as claimed in Claim 19, wherein said ester is the methyl ester.

22. A process as claimed in Claim 19, wherein said salt is an alkali metal salt.

23. A process as claimed in Claim 19, wherein said salt is a sodium salt.

24. A process as claimed in Claim 15, wherein said microorganism is:

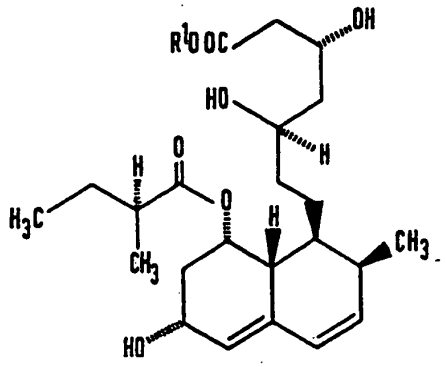
Absidia coerulea
Cunninghamella echinulata
Syncephalastrum racemosum
Mucor hiemalis f. hiemalis
Mucor bacilliformis
Mucor circinelloides f. circinelloides
Mucor hiemalis f. corticolus
Mucor dimorphosporus
Mucor fragilis
Mucor genevensis
Mucor globosus
Mucor circinelloides f. griseo-cyanus
Mucor heterosporus
Mucor spinescens
Pycnoporus coccineus
Rhizoctonia solani

Syncephalastrum nigricans.

25. A process as claimed in Claim 15, wherein said microorganism is:
Absidia coerulea IFO—4423
Cunninghamella echinulata IFO—4445
Cunninghamella echinulata IFO—4444
Cunninghamella echinulata ATCC—9244
Syncephalastrum racemosum IFO—4814
Syncephalastrum racemosum IFO—4828
Mucor hiemalis f. hiemalis IFO—5834
Mucor hiemalis f. hiemalis IFO—5303
Mucor hiemalis f. hiemalis IFO—5304
Mucor hiemalis f. hiemalis IFO—8567
Mucor hiemalis f. hiemalis IFO—8449
Mucor hiemalis f. hiemalis IFO—8448
Mucor hiemalis f. hiemalis IFO—8565
Mucor hiemalis f. hiemalis CBS—117.08
Mucor hiemalis f. hiemalis CBS—109.19
Mucor hiemalis f. hiemalis CBS—200.28
Mucor hiemalis f. hiemalis CBS—242.35
Mucor hiemalis f. hiemalis CBS—110.19
Mucor hiemalis f. hiemalis CBS—201.65
Mucor bacilliformis NRRL—2346
Mucor circinelloides f. circinelloides IFO—4554
Mucor circinelloides f. circinelloides IFO—5775
Mucor hiemalis f. corticolus NRRL—12473
Mucor dimorphosporus IFO—4556
Mucor fragilis CBS—236.35
Mucor genevensis IFO—4585
Mucor globosus NRRL—12474
Mucor circinelloides f. griseo-cyanus IFO—4563
Mucor heterosporus NRRL—3154
Mucor spinescens IAM—6071
Pycnoporus coccineus NRRL—12476
Rhizoctonia solani NRRL—12477
Syncephalastrum nigricans NRRL—12478
Syncephalastrum nigricans NRRL—12479 or
Syncephalastrum nigricans NRRL—12480.

26. A process as claimed in Claim 15, wherein said microorganism is:
Mucor hiemalis f. hiemalis
Mucor circinelloides f. circinelloides
Mucor fragilis
Mucor genevensis
Mucor circinelloides f. griseo-cyanus
Pycnoporus coccineus or
Rhizoctonia solani.

27. A process as claimed in Claim 15, wherein there is prepared a compound of formula (VI):



(VI)

(wherein
R^1 represents a hydrogen atom or a C_1—C_5 alkyl group), a pharmaceutically acceptable salt of the acid wherein R^1 represents a hydrogen atom, or a compound of formula (VII):